Journal of Pharmaceutical and Biomedical Analysis 78–79 (2013) 45–51

Contents lists available at SciVerse ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Identification of a 14 kDa endocan fragment generated by cathepsin G, a novel circulating biomarker in patients with sepsis

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ARTICLE INFO

Article history:
Received 13 September 2012
Received in revised form 23 January 2013
Accepted 25 January 2013
Available online 9 February 2013

Keywords:
Sepsis
Endothelium
Endocan
Cathepsin G
Biomarker
Immunoassay

ABSTRACT

Severe septic syndrome, which is the most prevalent and lethal cause of acute respiratory distress syndrome, remains one of the most frequent causes of admission and death in intensive care units (ICU). Inflammatory phenomenon leading to severe sepsis are multiple and not yet completely understood. The main target damage during severe sepsis is the endothelium. Endocan, specifically secreted by activated-pulmonary vascular endothelial cells, is thought to play a key role in the control of the lung inflammatory reaction. A recent clinical investigation found that a low plasma endocan level was predictive of respiratory failure. In this study, the hypothesis that low levels of endocan may result from proteolysis was tested. We demonstrate that cathepsin G (CG), neutrophil elastase (NE), and to a lesser extent proteinase 3 (PR3), degrade endocan. Interestingly, a novel endocan peptide fragment of 14 kDa, named p14, was identified, resulting from the specific cleavage of endocan by CG, corresponding to the N-terminal 111–116 amino acids of the endocan polypeptide. An immunoassay specific for p14 endocan fragment was then developed, and revealed increased plasma levels of p14 in 20 out of 55 severe septic patients, ranging from 0.52 to 10.40 ng/mL versus undetectable p14 in plasma from 32 control subjects ($p = 0.0011$). No correlations were found between p14 and endocan blood levels in severe septic patients. Taken together, the p14 endocan fragment represents a novel interesting biomarker which could participate to the pathogenesis of sepsis.

1. Introduction

Sepsis remains one of the most frequent cause of admission and death in intensive care units (ICU) [1–4]. It is well known that an increase in the number of failing organs during the first 48 h following admission to an ICU is a good indicator of mortality in septic patients [5]. Therefore, the early diagnosis of organ dysfunction progression to multiple organ failure (MOF) is one of the primary goals in the treatment of septic patients [6]. Endothelial injury is recognized to be one major initiating component of MOF, and is critical for patient outcome [7–9]. Among the various biomarkers of endothelial dysfunction currently under experimental investigation, our laboratory has focused on a novel biomarker endocan being produced by lung capillaries [10–13].

Early studies have demonstrated that blood levels of endocan are increased in patients with severe sepsis, constituting a bad prognosis signature [12,14]. More recently, the levels of blood endocan have been reported to predict the occurrence of acute lung injury (ALI) in the context of polytrauma [15]. In particular, the authors found that patients with high endocan levels did not develop ALI. Inversely, low endocan levels were predictive of a high risk of ALI [15]. This unexpected observation led us to hypothesize that during severe sepsis endocan proteolysis could occur, leading to exacerbated leukocyte diapedesis, and triggering MOF.

Here, cathepsin G was found to generate a unique endocan degradation profile. Interestingly, a novel 14 kDa endocan fragment named p14 was identified. A specific immunoassay for p14 was then developed and evidenced substantial increase of p14 in blood from septic patients which did not correlate with endocan. Our

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http://dx.doi.org/10.1016/j.jpba.2013.01.035

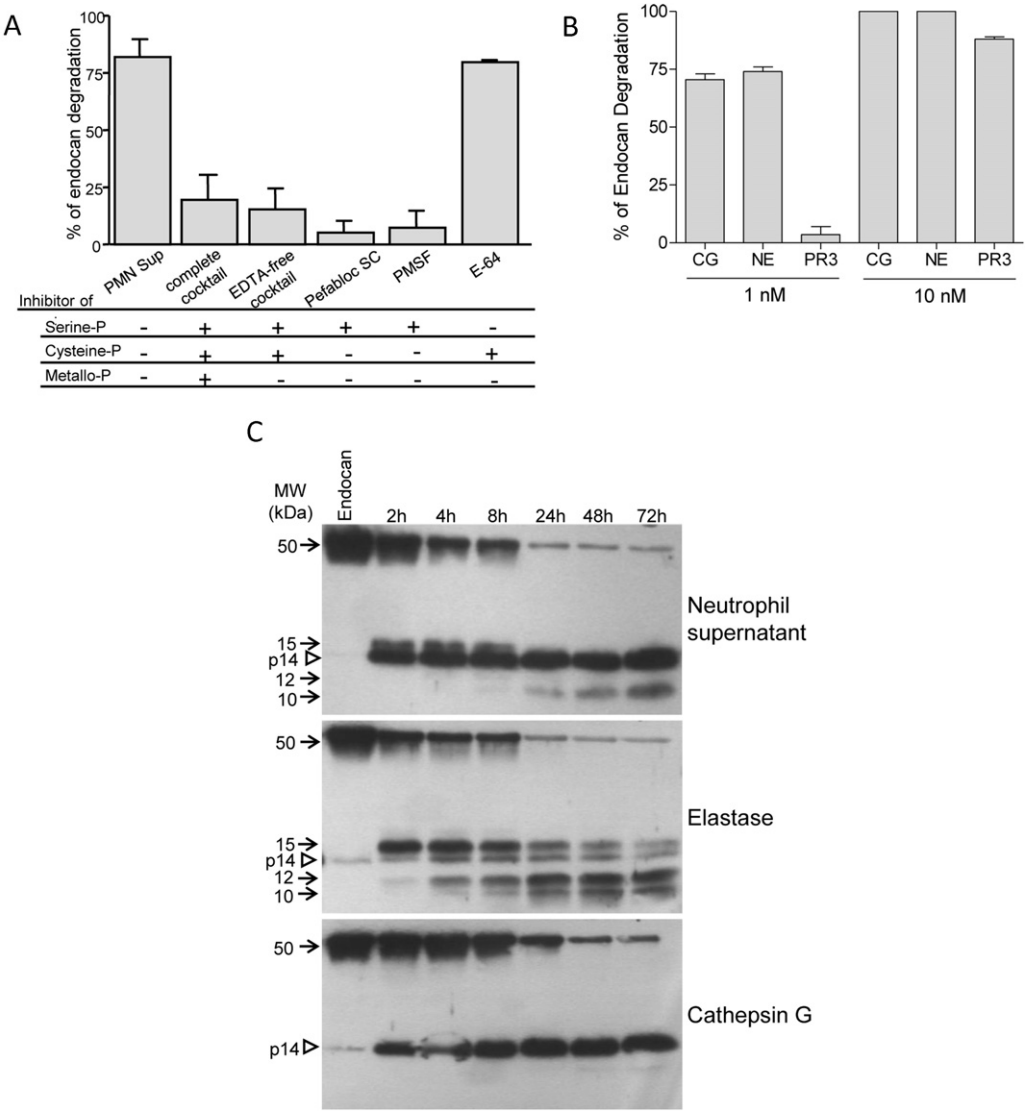

Fig. 1. Neutrophil-derived cathepsin G generates a major 14 kDa endocan fragment. (A) Effect of protease inhibitors on neutrophil-induced endocan degradation. The results are expressed in % of endocan degradation = [1 − residual endocan/input endocan] × 100. (B) Effect of CG, NE and PR3. Results are expressed in % of endocan degradation. (C) Kinetics of endocan degradation induced by neutrophil supernatant, elastase or cathepsin G.

results suggest that p14 represents the main catabolite of endocan induced by neutrophil proteases, opening new insights in the diagnosis and the follow-up of MOF during sepsis.

2. Materials and methods

2.1. Reagents

All the anti-endocan monoclonal antibodies (mAb), purified endocan, and non glycanable endocan (S137A/E) were provided from Lunginnov (Lille, France). The MEP14 mAb maps the peptide 159–165, relevant for immunoelimination of full length endocan [16]. The MEP21 mAb maps the peptide 60–79, relevant for western blot. The MEC15 mAb maps the same epitope in its native conformation relevant for ELISA or immunoprecipitation. The MEC36 mAb maps an epitope included in the peptide 80–130 in its native form also relevant for ELISA or immunoprecipitation [16].

2.2. Isolation of neutrophils

Neutrophils were isolated from blood using Ficoll Paque Plus gradient (GE Healthcare Bio-Sciences) followed by a hypotonic saline lysis of erythrocytes of the granulocyte pellet. Secretion of neutrophil proteases was induced by 10 nM phorbol myristate acetate for 3 h at 37 °C in HBSS without calcium or magnesium (Gibco).

2.3. Enzymatic degradation of endocan

Recombinant endocan (0.11 μM) was incubated in PBS containing 0.01% human serum albumin (HSA) and 10% (v/v) neutrophil supernatant. The purified proteases used for endocan degradation experiment in Fig. 1B are titrated enzymes generously given by Pr. Francis Gauthier (INSERM U618, Tours, France). Each protease was incubated at 1 nM or 10 nM with recombinant endocan for 1 h at 37 °C, i.e. at a protease:endocan ratio of 1:100 or 1:10. For all the other experiments, purified cathepsin G (Sigma, catalog number C4428) and elastase (Sigma, catalog number E8140) were used at 0.33 nM and 3.3 nM, respectively. For the protease inhibition experiment, the protease inhibitor cocktail with or without EDTA (Roche), 1 mM PMSF (Sigma), 1 mM Pefabloc SC (Invitrogen), 20 μM E-64 (Invitrogen), were pre-incubated with proteases for 2 h at 37 °C before addition of endocan and HSA. The mixtures were then incubated for 24 h.